Genome-wide association study with imputed whole-genome sequence variants including large deletions for female fertility in 3 Nordic dairy cattle breeds

Md Mesbah-Uddin,^{1,2*} Bernt Guldbrandtsen,^{1†} Aurélien Capitan,^{2,3} Mogens Sandø Lund,¹ Didier Boichard,² and Goutam Sahana^{1‡}

¹Center for Quantitative Genetics and Genomics, Aarhus University, 8830 Tjele, Denmark

²Génétique Animale et Biologie Intégrative (GABI), Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), AgroParisTech, Université Paris-Saclay, 78350 Jouy-en-Josas, France

³Alice, 75595 Paris, France

ABSTRACT

Fertility is an economically important trait in livestock. Poor fertility in dairy cattle can be due to loss-of-function variants affecting any essential gene that causes early embryonic mortality in homozygotes. To identify fertility-associated quantitative trait loci, we performed single-marker association analyses for 8 fertility traits in Holstein, Jersey, and Nordic Red Dairy cattle using imputed whole-genome sequence variants including SNPs, indels, and large deletion. We then performed stepwise selection of independent markers from GWAS loci using conditional and joint association analyses. From single-marker analyses for fertility traits, we reported genome-wide significant associations of 30,384 SNPs, 178 indels, and 3 deletions in Holstein; 23,481 SNPs, 189 indels, and 13 deletions in Nordic Red; and 17 SNPs in Jersey cattle. Conditional and joint association analyses identified 37 and 23 independent associations in Holstein and Nordic Red Dairy cattle, respectively. Fertility-associated GWAS loci were enriched for developmental and cellular processes (Gene Ontology enrichment, false discovery rate < 0.05). For these quantitative trait loci regions (top marker and 500 kb of surrounding regions), we proposed several candidate genes with functional annotations corresponding to embryonic lethality and various fertility-related phenotypes in mouse and cattle. The inclusion of these top markers in future releases of the custom SNP chip used for genomic evaluations will enable their validation in

independent populations and improve the accuracy of genomic predictions.

Key words: genome-wide association study, fertility, deletion, dairy cattle

INTRODUCTION

Over the years, GWAS have provided a better understanding of underlying genetic architectures for many economical traits in dairy cattle. These traits include milk yield (Iso-Touru et al., 2016), milk composition (Gebreyesus et al., 2019; Sanchez et al., 2019), milking speed (Jardim et al., 2018; Marete et al., 2018), clinical mastitis (Kadri et al., 2015; Cai et al., 2018), body conformation (Abo-Ismael et al., 2017), stature (Bouwmans et al., 2018), and more. Female fertility is an economically important trait, which, before the advent of genomic selection, was difficult to improve by selection due to its very low heritability. The use of GWAS for female fertility provided an opportunity to detect QTL (Höglund et al., 2014, 2015a,b) and to include these QTL in genomic prediction (Brøndum et al., 2015).

However, the accuracy of prediction for female fertility is lower compared with production traits (Brøndum et al., 2015). There is a potential to identify QTL or markers in strong linkage disequilibrium (LD) with QTL using GWAS for female fertility to use the QTL information in breeding value prediction. Two ways to increase the power of GWAS in mapping populations of fixed size are (1) to consider additional classes of genetic variants in addition to SNPs such as small indels and various types of structural variations, and (2) to impute SNP chip genotyping data to the whole-genome sequence.

As for structural variants, particularly large chromosomal deletions (e.g., >50 bp in size) are potential candidates for loss of function where deletion of an essential gene or part of it could be lethal and cause

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*Current address: Natarajan Lab, Medical and Population Genetics Broad Institute of MIT and Harvard, Cambridge, MA 02142.

†Current address: Department of Veterinary and Animal Sciences, University of Copenhagen, Frederiksberg C 1870, Denmark.

‡Corresponding author: goutam.sahana@qgg.au.dk

embryonic mortality in homozygotes. Known causal deletions include *FANCI* deletion causing brachyspina in Holstein (Charlier et al., 2012), *RNASEH2B* deletion causing embryonic lethality (Kadri et al., 2014), and a ~525-kb deletion (encompassing *BTBD9*, *GLO1*, and *DNAH8*) responsible for stillbirth (Sahana et al., 2016; Mesbah-Uddin et al., 2018) in Nordic Red cattle. Furthermore, deletions in cattle were also enriched for health- and fertility-related QTL in Cattle QTLdb (Hu et al., 2016; Mesbah-Uddin et al., 2018), which underscore the functional and biological relevance of these variants in fitness-related traits.

To include deletions in addition to SNPs and indels in gene mapping and prediction of breeding values in cattle, we previously performed genome-wide mapping of deletions in 175 whole-genome sequence (WGS) animals, 67 Holstein (HOL), 81 Nordic Red (RDC), and 27 Jersey (JER) cattle, and identified ~8,500 variants ranging from 199 bp to 773 kb in size (Mesbah-Uddin et al., 2018). Later, we imputed these deletions along with WGS SNPs and indels, with an average imputation accuracy squared Pearson correlation (r^2) > 0.9, in a cohort of 13,000 bulls from HOL, RDC, and JER genotyped using medium- and high-density SNP array chip (Mesbah-Uddin et al., 2019). In this study, we performed GWAS for 8 fertility traits in HOL, RDC, and JER using imputed WGS variants including SNPs, indels, and deletions. We hypothesized that inclusion of indels and large deletions in addition to whole-genome single nucleotide variants could identify candidate genes for female fertility traits in dairy cattle.

METHODS

No animal handling or animal experiment was conducted for this study; therefore, approval from institutional animal care and use was not necessary.

Genotypes and Phenotypes

In an earlier study, using a multibreed WGS reference panel of 772 animals that included 548 HOL, 132 RDC, and 92 JER animals, we performed imputation from the Illumina BovineSNP50 (50k) to WGS SNPs, small indels, and deletions in a cohort of ~13,000 bulls [6,371 HOL, 4,955 RDC, and 1,640 JER; for details see Mesbah-Uddin et al. (2018, 2019)]. In short, for the imputation reference panel, SNPs and indels were identified from 175 WGS samples from the Nordic sequence data (Brøndum et al., 2014) and 597 WGS samples from the Run-6 of the 1000 Bull Genomes Project (Daetwyler et al., 2014) using the GATK v1.6 (McKenna et al., 2010) and SAMtools 0.1.18 mpileup (Li et al., 2009) software,

respectively. Additionally, chromosomal deletions (199 bp–773 kb) were mapped and genotyped in the 175 Nordic WGS samples (Mesbah-Uddin et al., 2018) using Genome STRiP software v2.00.1678 (Handsaker et al., 2011). Subsequently, deletion genotyping was extended to the remaining 597 WGS samples of the reference panel using the read-depth data from the variant call format file in a Gaussian mixture model-based genotyping approach (Mesbah-Uddin et al., 2019).

Imputation was performed in 2 steps using the multibreed reference panel. First, we imputed 9,932 animals (5,371 HOL, 3,723 RDC, and 838 JER) from the 50k genotypes to the BovineHD BedChip genotypes (777k) using a 777k haplotype reference panel of 3,375 animals (1,215 HOL, 1,325 RDC, and 835 JER). Second, using the multibreed WGS reference panel, the target ~13k samples were imputed from the 777k genotypes to WGS SNPs, small indels, and deletions. Here, genotype phasing was performed using a combination of Beagle v4.1.274 (Browning and Browning, 2016) and SHA-PEIT v2.837 software (Delaneau et al., 2013, 2014). Imputation was performed using Minimac3 v2.0.1 (Das et al., 2016). Overall, average imputation accuracies (Minimac r^2) were 0.93 for SNPs, 0.90 for small indels, and 0.90 for deletions (Mesbah-Uddin et al., 2019). In this study, we used the imputed WGS data set of 13k animals to perform GWAS for female fertility in HOL, RDC, and JER (Table 1).

From the above-mentioned imputation data set (which had several filters for low quality markers including deviation from Hardy-Weinberg proportion, $P \leq 1 \times 10^{-5}$), we considered markers with minor allele frequency >1% and Minimac r^2 value ≥ 0.1 for GWAS. At this minor allele frequency and Minimac r^2 , the genotype data set comprised 12,174,287 markers in HOL (12,076,004 SNPs; 94,581 indels; 3,702 deletions), 12,855,372 markers in RDC (12,748,285 SNPs, 102,843 indels, 4,244 deletions), and 10,135,228 markers in JER (10,057,293 SNPs; 74,829 indels; 3,106 deletions).

The phenotypic data in each breed were deregressed breeding values of AI bulls (VanRaden and Wiggans, 1991; Garrick et al., 2009) for 8 fertility traits as follows: number of inseminations per conception in heifers, number of inseminations per conception in cows (AISc), interval (number of days) from calving to first insemination in cows (ICF), interval (number of days) from first to last insemination in heifers, interval from first to last insemination in cows (IFLc), fertility index (FI), nonreturn rate in heifers, and nonreturn rate in cows (NRRc); for details, see NAV (2013). Deregressed proofs are a proxy of the average performance of bulls' daughters, adjusted for nongenetic effects and breeding values of their dams. Summary of these phenotypes

Table 1. Summary of female fertility traits (deregressed breeding values) analyzed

Trait ¹	Holstein			Nordic Red Dairy Cattle			Jersey		
	Number	Mean (SD)	Range	Number	Mean (SD)	Range	Number	Mean (SD)	Range
AISe	5,342	100.30 (11.38)	65–142	4,291	102.57 (10.56)	73–136	1,179	99.54 (10.11)	69–126
AISh	5,487	100.06 (10.00)	72–131	4,345	95.27 (12.62)	59–132	1,204	100.22 (12.70)	60–133
ICF	5,342	98.38 (11.19)	65–131	4,299	99.21 (10.20)	69–130	1,186	103.96 (10.9)	75–135
IFLc	5,361	98.1 (12.09)	60–146	4,309	101.63 (10.17)	73–134	1,192	99.74 (11.53)	65–136
IFLh	5,478	98.75 (10.97)	68–133	4,341	95.18 (11.64)	62–129	1,199	101.66 (11.37)	68–134
FI	5,577	98.86 (10.77)	68–142	4,471	100.44 (9.67)	74–129	1,211	101.14 (11.42)	69–131
NRRc	5,353	100.77 (10.21)	72–135	4,309	102.34 (10.56)	73–136	1,189	97.10 (9.67)	67–126
NRRh	5,487	101.65 (11.38)	70–137	4,351	96.76 (10.98)	65–129	1,207	99.95 (10.86)	65–130

¹AISe = number of inseminations per conception in cows; AISh = number of inseminations per conception in heifers; ICF = interval (number of days) from first to last insemination in heifers; FI = fertility index; NRRc = nonreturn rate in cows; NRRh = nonreturn rate in heifers.

was presented in Table 1. The final data set included phenotypes (breeding values) and imputed genotypes for 5,596 HOL, 4,507 RDC, and 1,215 JER bulls.

Genome-Wide Association Study

We performed single-marker association analyses using the following mixed linear model:

$$\mathbf{y} = \mathbf{1}_n\mu + b\mathbf{x} + \sum_{i=1}^{10} c_i \mathbf{PC}_i + \mathbf{g} + \boldsymbol{\epsilon},$$

where $\mathbf{y}$ was a vector of phenotypic records for fertility traits; $\mathbf{1}_n$ was a vector of ones; μ was the mean of the given trait; b was the additive allele substitution effect of the candidate marker to be tested; $\mathbf{x}$ was a vector of allele dosages (coded as 0, 1, or 2), $\mathbf{PC}_i$ was a vector of animals' scores for principal components of genetic ancestry i [first 10 principal components ($\mathbf{PC}$) were considered, calculated from a random set of 10% markers selected from the 29 autosomes]; c_i was the regression coefficient for score i ; $\mathbf{g}$ was a vector of polygenic effects with $\mathbf{g} \sim N(0, \mathbf{G}\sigma_g^2)$, where $\mathbf{G}$ was the genomic relationship matrix calculated using the 777k markers without the test chromosome [leave-one-chromosome-out (LOCO) approach]; σ_g^2 was the additive genetic variance explained by the 777k markers in LOCO approach; $\boldsymbol{\epsilon}$ was the vector of residual effect with $\boldsymbol{\epsilon} \sim N(0, \mathbf{I}\sigma_\epsilon^2)$, where $\mathbf{I}$ was an $n \times n$ identity matrix and σ_ϵ^2 was the residual variance. Differences in reliabilities of deregressed proofs were ignored as we used deregressed breeding values from progeny-tested bulls. The analysis was performed using GCTA version 1.92.1 (Yang et al., 2011a). Any association with the trait of interest was considered statistically significant at $P < 5 \times 10^{-8}$, considering 1 million independent tests at 5% level of significance.

We estimated the genomic inflation factor, λ , for the set of single-marker GWAS P -values using “median(qchisq(1 - $\mathbf{P}$, 1))/qchisq(0.5, 1)” in R (R v4.0; cran.r-project.org), where $\mathbf{P}$ is a vector of GWAS P -values.

Conditional and Joint Association Analysis

Next, to refine the analysis within each QTL region and account for LD, we performed stepwise model selection using genome-wide complex trait analysis (GCTA)-conditional and joint association analysis (COJO) (Yang et al., 2012). For each chromosome, from the single-marker associations at $P < 5 \times 10^{-8}$, we selected the marker with lowest association P -value

(top marker) and reran the GWAS for the chromosome including the genotype of the top marker and first 10 PC as fixed effects, and the LOCO-genomic relationship matrix as random effect. We used an LD threshold of 0.80 to identify independent marker(s) and assumed complete independence for markers at least 10 Mb away from a conditional marker. After each iteration, the marker with lowest P -value was selected when it was below the GWAS threshold of 5×10^{-8} and was added to the conditional marker list for the next iteration. When there were multiple markers with same (lowest) P -value, the first marker based on chromosome position was arbitrarily chosen. Finally, for each chromosome, all selected markers on the chromosome were fitted simultaneously.

Annotation and Enrichment Analysis

We used the *Bos taurus* reference genome assembly UMD3.1 (Zimin et al., 2009; Elsik et al., 2016) throughout this study. To identify candidate genes within a QTL, we used the *Ensembl* (release 94) database (<https://ftp.ensembl.org/pub/release-94>; Zerbino et al., 2018) and manually inspected 1 Mb genomic region centered on the top marker (i.e., top marker 500 kb of the surrounding region). From *Ensembl*, we retrieved annotations for genes and known phenotypes associated with those genes. However, when multiple genes were located within an interval, for reporting, we prioritized genes with records of fertility- and fitness-related phenotypes in Online Mendelian Inheritance in Animals (OMIA; www.oma.org), Online Mendelian Inheritance in Man (OMIM; <http://www.omim.org/>), mouse (<http://www.informatics.jax.org/> and <https://www.mousephenotype.org/>), and rat (<https://rgd.mcw.edu/wg/>).

The Gene Ontology (GO) analysis was performed for FI-associated variants at $P < 5 \times 10^{-8}$ in HOL, or RDC, or nominally associated at $P < 5 \times 10^{-5}$ in JER. We extracted genes within 500 kb upstream and 500 kb downstream of the FI-associated variants from *Ensembl* UMD3.1 gene annotation (http://ftp.ensembl.org/pub/release-94/gtf/bos_taurus/Bos_taurus.UMD3.1.94.chr.gtf.gz). The g:Profiler web server (Raudvere et al., 2019) was used for GO enrichment analysis, and GO terms at false discovery rate < 0.05 were reported.

Additionally, we also used R base package (v4.0.2), qqman (Turner, 2018), ggplot2, and VennDiagram (Chen and Boutros, 2011) for plotting.

RESULTS AND DISCUSSION

We performed single-marker association analysis for 8 female fertility traits (Table 1) in HOL, RDC, and

JER cattle using imputed WGS SNPs, indels, and deletions. The GWAS Manhattan plots for 8 traits in the 3 breeds are presented in Figures 1, 2, and 3, and the summary of variants that reached genome-wide significant threshold of $P < 5 \times 10^{-8}$ is presented in Table 2.

Association test statistics were inflated when we only accounted for relatedness using the genomic relationship matrix derived from the 777k markers in a LOCO approach (Table 3). Therefore, following Jiang et al. (2019), we adjusted for both population stratification using the first 10 PC as fixed effects, and relationships among animals using the LOCO approach. Genomic inflation rates (λ) before and after fitting the PC are presented in Table 3. We observed a decrease in λ values after the inclusion of PC as covariates, although λ was still very far from 1 under the assumption of the distribution of test statistics under the null hypothesis. However, when a trait is under polygenic inheritance [i.e., multiple (small effect) causal variants underlie the trait variation (also known as infinitesimal model)], a substantial inflation of GWAS test statistics is expected even in the absence of population structure (Yang et al., 2011b). The extent of this inflation is governed by several factors, namely, heritability of the trait, study sample size, pattern of LD, and number of causal variants [for detail, see Yang et al. (2011b)]. The genomic inflation seen in this study despite the correction for population stratification and relatedness could be explained by the nature of polygenic inheritance of the fertility traits we analyzed (e.g., several nonzero effect genetic variants in Bayesian mixture models as reported by Brøndum et al., 2015). The phenotypes used here are deregressed breeding values for bulls with high reliability. The long-range LD pattern seen in the study population (de Roos et al., 2008; van den Berg et al., 2016) could also explain inflation of λ values observed in this study. Among the 3 breeds, HOL had the largest sample size, and thus the highest power to identify QTL; we also observed the highest λ values in HOL. However, the adjustment for both population stratification and relatedness could be very conservative; nonetheless, we expect that the test statistics will be robust against spurious associations and false discoveries (Jiang et al., 2019).

Fertility-Associated Loci Are Enriched for GO Terms Related to Cellular and Developmental Processes

We performed GO enrichment using genes within a 500-kb surrounding area of FI-associated loci in HOL, RDC, and JER. Interestingly, only 22 genes (within chromosome 13: 51–55 mb) were common among the 3 breeds (Figure 4a). Despite limited overlap, gene-set enrichment analysis showed that genes near the FI-

Figure 1. Manhattan plots of single-marker GWAS for 8 female fertility traits in Holstein cattle. Each dot represents 1 whole-genome sequence (WGS) marker; dashed horizontal line is GWAS threshold of 5×10^{-8} . AISh = number of inseminations per conception in heifers; AISc = number of inseminations per conception in cows; ICF = interval (number of days) from calving to first insemination (in cows); IFLh = interval (number of days) from first to last insemination in heifers; IFLc = interval (number of days) from first to last insemination in cows; FI = fertility index; NRRh = nonreturn rate in heifers; NRRc = nonreturn rate in cows.

Figure 2. Manhattan plots of single-marker GWAS for 8 female fertility traits in Nordic Red Dairy cattle. Each dot represents 1 whole-genome sequence (WGS) marker; dashed horizontal line is GWAS threshold of 5×10^{-8} . AISh = number of inseminations per conception in heifers; AISc = number of inseminations per conception in cows; ICF = interval (number of days) from calving to first insemination (in cows); IFLh = interval (number of days) from first to last insemination in heifers; IFLc = interval (number of days) from first to last insemination in cows; FI = fertility index; NRRh = nonreturn rate in heifers; NRRc = nonreturn rate in cows.

Figure 3. Manhattan plots of single-marker GWAS for 8 female fertility traits in Jersey cattle. Each dot represents 1 whole-genome sequence (WGS) marker; dashed horizontal line is GWAS threshold of 5×10^{-8} . AISH = number of inseminations per conception in heifers; AISc = number of inseminations per conception in cows; ICF = interval (number of days) from calving to first insemination (in cows); IFLh = interval (number of days) from first to last insemination in heifers; IFLc = interval (number of days) from first to last insemination in cows; FI = fertility index; NRRh = nonreturn rate in heifers; NRRc = nonreturn rate in cows.

Table 2. Summary of single-marker association results (markers with P -value $< 5 \times 10^{-8}$)

Trait ¹	Holstein	Nordic Red Dairy Cattle	Jersey
AISh	583	10,004	0
AISc	2,012	16,089	0
ICF	21,539	280	8
IFLh	932	7,302	0
IFLc	7,645	16,289	1
FI	11,091	14,916	0
NRRh	1,057	8,036	0
NRRc	2,008	9,182	8

¹AISh = number of inseminations per conception in heifers; AISc = number of inseminations per conception in cows; ICF = interval (number of days) from calving to first insemination (in cows); IFLh = interval (number of days) from first to last insemination in heifers; IFLc = interval (number of days) from first to last insemination in cows; FI = fertility index; NRRh = nonreturn rate in heifers; NRRc = nonreturn rate in cows.

associated GWAS loci are enriched for developmental and cellular processes (false discovery rate < 0.05) in HOL, RDC, and JER (Figure 4b–d). Furthermore, majority of the GO enrichments were in similar molecular, cellular, and biological processes in the 3 breeds (Supplemental Tables S1–S3; <https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021). Although our GWAS was underpowered, especially for JER, this GO enrichment highlighted that common biological pathways may be involved in female fertility in dairy cattle. This pathway sharing across-breeds showed that there is a potential to use multibreed reference in predicting fertility in dairy cattle, where substantial gains in accuracy could be achieved for breeds with numerically small reference populations.

Deletions Associated With Female Fertility Traits

In HOL, 3 deletions were significantly associated ($P < 5 \times 10^{-8}$) with ICF, FI, and NRRc (Table 4). We identified 3 candidate genes, *ACSS1*, *CFAP61*, and

HELLS, within 500 kb upstream and 500 kb downstream of these deletions, that are known to be associated with embryonic lethality, premature death, and male infertility in mouse (Supplemental Table S4; <https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021). A recent study also showed that *CFAP61* plays a vital role in male fertility in humans (Liu et al., 2021).

In RDC, 13 deletions reached genome-wide significance; associated phenotypes included 7 fertility traits (Table 4). The strongest association was observed for a previously known lethal deletion on chromosome 12 located between position 20,100,648 and 20,763,119 bp (structural variant id esv4015629, $P = 3.5 \times 10^{-42}$). We found that esv4015629 was significantly associated with 7 fertility traits, in agreement with results reported by Kadri et al. (2014). However, we did not find any association between esv4015629 and ICF. It is worth mentioning that ICF is related to estrous cycle (pre-pregnancy), and thus is not affected by early embryo losses, whereas the 7 other associated traits are related to pregnancy success. Several traits had multiple associated deletions. Three traits, FI, IFLc, and AISc, had strong genetic correlations (correlation of 0.98 between FI vs. IFLc, and 0.89 between FI vs. AISc). They shared associations with 7 deletions (Table 4). Several genes within these deletions have known fertility-related phenotypes, including embryonic lethality in mice (Supplemental Table S4).

However, no deletion reached genome-wide significance for female fertility traits in JER. The top 5 associations included esv4014584 [chromosome (Chr)13:50,733,796–50,735,009; associated with ICF, $P = 3.2 \times 10^{-6}$], esv4018559 (Chr6:33,504,998–33,506,227; associated with AISc, $P = 3.4 \times 10^{-6}$), esv4019122 (Chr6:33,738,269–33,738,743; associated with AISc, $P = 3.4 \times 10^{-6}$), esv4017388 (Chr1:145,570,019–145,570,346; associated with NRRc, $P = 8.5 \times 10^{-6}$), and esv4017421 (Chr3:7,140,763–7,141,120; associated with

Table 3. Genomic inflation rate, λ , before and after correcting for population stratification using first 10 principal components (PC)

Trait ¹	Holstein		Nordic Red Dairy Cattle		Jersey	
	Without PC	With PC	Without PC	With PC	Without PC	With PC
AISh	1.76	1.65	1.41	1.37	1.39	1.29
AISc	2.04	1.83	1.56	1.50	1.31	1.24
ICF	2.38	2.19	1.63	1.57	1.36	1.29
IFLh	1.93	1.81	1.42	1.37	1.37	1.29
IFLc	2.24	2.02	1.59	1.53	1.32	1.25
FI	2.26	2.05	1.59	1.52	1.33	1.27
NRRh	1.99	1.84	1.41	1.38	1.37	1.29
NRRc	2.20	1.98	1.53	1.48	1.39	1.28

¹AISh = number of inseminations per conception in heifers; AISc = number of inseminations per conception in cows; ICF = interval (number of days) from calving to first insemination (in cows); IFLh = interval (number of days) from first to last insemination in heifers; IFLc = interval (number of days) from first to last insemination in cows; FI = fertility index; NRRh = nonreturn rate in heifers; NRRc = nonreturn rate in cows.

Figure 4. Gene-set enrichment analysis. (a) Venn diagram of overlapping genes among fertility index (FI)-associated loci (500 kb upstream and 500 kb downstream) in Holstein (HOL), Nordic Red Dairy cattle (RDC), and Jersey (JER). The FI GWAS P -value thresholds of 5×10^{-8} in HOL and RDC, and 5×10^{-5} in JER were considered for gene selection. There are 22 genes (in chromosome 13:51–55 mb) common among FI-associated loci in the 3 dairy breeds. (b–d) Manhattan plot depicting Gene Ontology (GO) terms enriched (false discovery rate < 0.05) in HOL (b), RDC (c), and JER (d). Top 8 GO terms are highlighted. The GO enrichment analysis was performed using g:Profiler webserver (<https://biit.cs.ut.ee/gprofiler/gost>). Full results of GO enrichment are presented in Supplemental Tables S1–S3 (<https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021). P_{adj} = adjusted P -value.

Table 4. Fertility-associated large deletions (P -value $< 5 \times 10^{-8}$)

Chromosome	Marker ID ¹	Position ²	Size of deletion	Trait ³	A1/A2 ⁴	Freq ⁵	b^6	SE ⁷	P^8	Nearby gene
Holstein cattle	esv4017813	13:42,945,993-42,946,953	961 bp	ICF	DEL/G	0.271	-1.050	0.186	1.7×10^{-8}	ACSS1
	esv4018227	13:40,037,936-40,039,382	2.45 kb	FI	DEL/C	0.482	-0.894	0.155	8.1×10^{-9}	CFAP61
	esv4015626	26:16,220,854-16,223,815	2.96 kb	NRRc	DEL/T	0.097	1.418	0.248	1.0×10^{-8}	HELLS
Nordic Red										
Dairy cattle	esv4013561	6:31,705,882-31,706,925	1.04 kb	NRRh	DEL/C	0.287	-1.341	0.242	3.0×10^{-8}	SMARCA1
	esv4018710	6:37,999,532-37,999,979	448 bp	NRRh, NRRc	DEL/C	0.352	-1.525	0.236	9.4×10^{-11}	ABCG2; PKD2
	esv4019275	6:39,473,114-39,473,611	498 bp	NRRc	DEL/A	0.389	1.260	0.215	4.9×10^{-9}	LCORL
	esv4011295	6:39,711,454-39,712,517	1.06 kb	AISc	DEL/A	0.423	-1.208	0.215	1.9×10^{-8}	—
	esv4014663	6:39,856,457-39,856,658	202 bp	NRRc, AISc	DEL/A	0.466	1.502	0.211	1.2×10^{-12}	—
	esv4019299	12:17163364-17163728	365 bp	FI, AISh, AISc, IFLh, IFLc,	DEL/G	0.250	-1.655	0.226	2.2×10^{-13}	HTR2A
12	esv4015629	12:20,100,648-20,763,119	662 kb	NRRh	DEL/G	0.137	-3.985	0.293	3.5×10^{-42}	RNASEH2B; GUCY1B2; FAM124A
12	esv4012561	12:20,570,478-20,571,118	641 bp	IFLh, IFLc, NRRh, NRRc	DEL/A	0.092	-2.010	0.323	5.0×10^{-10}	GUCY1B2; RNASEH2B
12	esv4017726	12:22,961,548-22,961,822	275 bp	IFLc, AISc, FI	DEL/A	0.435	1.394	0.207	1.8×10^{-11}	LHFPL6; FREM2
12	esv4013857	12:35,299,066-35,299,339	274 bp	IFLc	DEL/T	0.127	-1.789	0.315	1.4×10^{-8}	SACS; FGF9
12	esv4015687	12:43,999,233-44,009,032	9.8 kb	IFLc, AISc, NRRc, FI	DEL/T	0.102	-2.255	0.331	9.8×10^{-12}	KLHL1
25	esv4014328	25:12,757,355-12,758,442	1.09 kb	FI, AISh, IFLh, AISc, IFLc	DEL/G	0.059	-2.432	0.401	1.3×10^{-9}	ERCC4; MRTFB
25	esv4014654	25:13,705,829-13,706,486	658 bp	IFLc, IFLh, FI, NRRh	DEL/A	0.050	-3.833	0.522	2.1×10^{-13}	RRN3

¹Marker ID = deletion IDs. These were from Database of Genomic Variants archive extracted from Ensembl release 94 (<http://ftp.ensembl.org/pub/release-94/>).²Position base pair positions in UMD3.1.³Traits included number of inseminations per conception in cows (AISc) and in heifers (AISh); interval (number of days) from calving to first insemination (ICF); interval from first to last insemination in cows (IFLc) or in heifers (IFLh); fertility index (FI); nonreturn rate in cows (NRRc) or in heifers (NRRh). When a deletion was associated with multiple traits, GWAS summary for trait with strongest signal (first trait in the corresponding row) was presented in the table.⁴A1/A2 = effect allele/other allele. DEL = deletion.⁵Freq = frequency of A1 allele.⁶ b = allele substitution effect.⁷Standard error of b estimates.⁸ P -value from single-marker GWAS.

nonreturn rate in heifers, $P = 1.8 \times 10^{-5}$). Among these 5 deletions, only esv4017388 was located within a QTL associated with NRRc (Figure 3h). Candidate genes for this locus include *ADARB1* (ENSBTAG00000017486, 52 kb upstream of esv4017388) and *POFUT2* (ENSBTAG00000007818, 467 kb downstream of esv4017388). In mice, homozygous mutation of *ADARB1* causes postnatal lethality (entry MGI: 891999 in the Mouse Genome Informatics database, <http://www.informatics.jax.org/>), whereas the inactivation of *POFUT2* causes embryonic lethality during organogenesis as well as lethality between implantation and somite formation (MGI: 1916863).

FI-Associated SNPs and Indels

FI-Associated Loci in HOL. In HOL, for the 8 fertility traits, 30,384 SNPs and 178 indels in total reached the significance threshold (Figure 1 and Table 2). Among these significant markers, COJO analysis identified 37 FI-associated independent loci [see Table 5 for the list of QTL and Supplemental Table S5 (<https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021) for candidate genes]. In addition to FI, we also performed COJO analyses for the 7 other traits and identified 217 independent loci associated with one or more of these fertility traits (Supplemental Table S6; <https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021). The top 3 FI loci in terms of significance were centered on variants rs434006863 on chromosome 13 ($P = 1.5 \times 10^{-22}$), rs380439408 on chromosome 24 ($P = 5.4 \times 10^{-14}$), and rs380495923 on chromosome 10 ($P = 2.0 \times 10^{-12}$). The candidate genes for rs434006863, rs380439408, and rs380495923 were *MPP7*, *CDH2*, and *TBPL2*, respectively. Homozygous mutation of *CDH2* causes embryonic lethality in mice [MGI: 88355], and female mice homozygous for a *TBPL2* knockout allele exhibit infertility due to impaired folliculogenesis [MGI: 2684058]. For details, see Supplemental Table S5.

FI-Associated Loci in RDC. In RDC, we identified 23,481 SNPs and 189 indels with genome-wide significant associations with fertility traits (Table 2). The COJO analysis identified 23 FI-associated independent GWAS signals, 9 of which were located on chromosome 12 (Table 6). The LD pattern (correlation) of these 9 lead markers is presented in Table 7. Functional annotations of the genes located within these QTL regions (top markers and 500 kb of the surrounding area) are presented in Supplemental Table S7 (<https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021). Interestingly, the candidate genes within these loci also have known fertility-related functions in cattle, and mouse (Supplemental Table S7). Overall,

the COJO analyses for the 8 traits resulted in 111 independent loci associated with either FI or different component traits (Supplemental Table S8; <https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021).

FI-Associated Loci in JER. Finally, for JER, 17 SNPs were significantly associated with either ICF, IFLc, or NRRc (Figure 3 and Table 2). After conditional analyses, we identified 2 independent GWAS loci each for ICF and NRRc, and 1 locus for IFLc (Supplemental Table S9; <https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021). However, our GWAS was underpowered to identify FI-associated loci in JER. In an earlier study, Höglund et al. (2015b) reported 6 FI-associated QTL with $P < 5.6 \times 10^{-9}$ for this JER population. The allele frequencies and the effect estimates (β) for the Bonferroni significant FI-associated SNPs from the Höglund et al. study was perfectly correlated ($r > 0.99$) with the corresponding results from this study (Supplemental Figure S1a,b; <https://doi.org/10.6084/m9.figshare.16910521.v2>; Mesbah-Uddin, 2021); however, on average, the β values were 1.8 times higher in the former study (Supplemental Figure S1b). Moreover, we did not observe significant association with FI in JER, though there were suggestive signals at those loci (Supplemental Figure S1c). Two factors, namely, sample size and control for genomic inflation, could explain this situation. In JER, we had about 4 times fewer samples compared with HOL and RDC, and thus lower QTL detection power. Sample size did not differ much between Höglund et al. and our study (1,225 vs. 1,211 here). However, control of genomic inflation was much more stringent in this study with a λ of 1.27 after correction versus 1.88 in Höglund et al. (2015b).

CONCLUSIONS

In this study, we identified several novel QTL for female fertility in Nordic dairy cattle. With our imputed data, we detected a previously observed deletion acting as a fertility QTL in RDC. Here, with imputed data, the GWAS signal for this QTL was stronger than in the previous report by Kadri et al. (2014). Few other deletions also reached the GWAS significant threshold but were not among the top signals of those loci. We hypothesize that these deletions could be potential causal variants for those loci, given that deletion of an essential gene could be lethal; however, this needs further validation. The majority of the candidate genes within the reported QTL regions had established fertility-related function in mice, illustrating the functional and biological relevance of these QTL for female fertility in dairy cattle. However, our analyses were limited

Table 5. Fertility index-associated GWAS loci in Holstein cattle

Chromosome	Top marker	Position ¹	A1/A2 ²	Freq ³	Single-marker analysis ⁴			Conditional and joint analysis ⁵			Nearby gene ⁶
					b	SE	P	b _j	SE _j	P _j	
1	rs137209107	68,642,776	A/G	0.143	-1.377	0.228	1.5×10^{-9}	-1.377	0.229	1.7×10^{-9}	MYLK
1	rs384339926	113,461,025	G/A	0.129	1.285	0.224	1.0×10^{-8}	1.285	0.225	1.1×10^{-8}	MME
2	rs207792133	23,885,602	C/T	0.131	-1.284	0.226	1.4×10^{-8}	-1.284	0.227	1.5×10^{-8}	RAPGEF4
2	rs43306949	50,233,158	G/A	0.159	-1.147	0.209	3.9×10^{-8}	-1.147	0.209	4.2×10^{-8}	SNRPD1
3	rs137588365	29,789,003	A/G	0.376	-0.997	0.159	3.6×10^{-10}	-0.997	0.160	4.1×10^{-10}	PHTF1
3	rs42253052	68,281,044	A/G	0.313	1.071	0.170	3.3×10^{-10}	1.071	0.171	3.8×10^{-10}	ST6GALNAC5
3	rs109192619	79,679,521	T/C	0.479	-0.902	0.159	1.4×10^{-8}	-0.902	0.159	1.6×10^{-8}	MGC137454; PDE4B; LEPR
4	rs135727662	47,510,332	A/G	0.489	0.954	0.153	4.8×10^{-10}	0.954	0.154	5.4×10^{-10}	NAMPT; SYPL1
4	rs385277008	65,156,839	G/A	0.172	1.270	0.207	7.9×10^{-10}	1.270	0.207	9.0×10^{-10}	PPP1R17
5	rs210968504	86,998,676	G/T	0.182	-1.127	0.205	3.7×10^{-8}	-1.127	0.205	4.0×10^{-8}	SOX5
6	rs110866176	93,466,329	C/G	0.449	-1.096	0.157	3.0×10^{-12}	-1.096	0.158	3.7×10^{-12}	SOWAHB; SHROOM3
7	rs473589185	93,099,110	T/G	0.060	-1.827	0.327	2.3×10^{-8}	-1.827	0.328	2.5×10^{-8}	ARRDC3
8	rs382943070	27,286,359	T/C	0.112	-1.491	0.245	1.2×10^{-9}	-1.491	0.246	1.4×10^{-9}	CNTLN
8	rs208577191	49,271,809	G/A	0.323	0.953	0.170	2.2×10^{-8}	0.953	0.171	2.4×10^{-8}	TMC1
10	rs380495923	68,425,703	T/A	0.320	-1.186	0.168	1.6×10^{-12}	-1.186	0.169	2.0×10^{-12}	TBPL2
10	rs134897493	81,159,267	T/C	0.354	-1.123	0.168	2.4×10^{-11}	-1.123	0.169	2.9×10^{-11}	ACTN1; ZFP36L1
11	rs134324803	23,631,737	G/C	0.242	-1.087	0.181	2.0×10^{-9}	-1.087	0.182	2.3×10^{-9}	—
11	rs209793386	42,987,167	T/G	0.168	1.307	0.209	3.9×10^{-10}	1.307	0.210	4.4×10^{-10}	BCL11A
13	rs384444008	25,178,930	G/T	0.214	1.040	0.190	4.6×10^{-8}	1.301	0.196	3.0×10^{-11}	KIAA1217
13	rs385034802	29,587,693	T/C	0.445	0.345	0.159	2.9×10^{-2}	0.990	0.168	4.2×10^{-9}	FAM107B
13	rs434006863	36,616,682	T/C	0.148	1.849	0.215	8.8×10^{-18}	2.192	0.224	1.5×10^{-22}	MPP7
13	rs136213414	51,160,223	A/G	0.157	-1.437	0.215	2.6×10^{-11}	-1.437	0.216	3.1×10^{-11}	HAO1
14	rs42685926	34,562,085	C/G	0.132	1.361	0.232	4.4×10^{-9}	1.361	0.233	4.9×10^{-9}	C14H8orf34
15	rs109077219	8,194,073	T/G	0.395	1.054	0.160	4.4×10^{-11}	1.054	0.161	5.2×10^{-11}	PGR
17	rs41853464	72,010,870	A/G	0.371	-0.999	0.162	7.0×10^{-10}	-0.999	0.162	7.9×10^{-10}	MORC2; SMTN
18	rs382112789	31,104,391	T/G	0.175	-1.166	0.208	2.0×10^{-8}	-1.166	0.208	2.2×10^{-8}	—
18	rs450967176	44,576,200	T/G	0.117	-1.717	0.244	1.9×10^{-12}	-1.717	0.245	2.3×10^{-12}	KCTD15; LSM14A
18	rs382522431	61,080,660	A/T	0.135	1.380	0.232	2.8×10^{-9}	1.380	0.233	3.1×10^{-9}	>10 genes within 500 kb
19	rs383235276	4,888,887	T/C	0.253	1.053	0.186	1.4×10^{-8}	1.053	0.186	1.6×10^{-8}	—
19	rs41911242	26,618,825	C/T	0.316	-0.978	0.166	3.8×10^{-9}	-0.978	0.166	4.2×10^{-9}	NLRP1
23	rs715807267	36,483,718	A/G	0.100	1.630	0.259	2.9×10^{-10}	1.630	0.259	3.3×10^{-10}	SOX4
24	rs380439408	29,556,826	A/C	0.233	-1.413	0.187	4.0×10^{-14}	-1.413	0.188	5.4×10^{-14}	CDH2
24	rs134963365	46,786,442	T/G	0.485	0.988	0.160	6.2×10^{-10}	0.988	0.160	7.1×10^{-10}	LOXHD1
24	rs379672347	62,207,595	G/T	0.248	-1.010	0.181	2.6×10^{-8}	-1.010	0.182	2.9×10^{-8}	KDSR; SERPINB5
25	rs459173018	2,987,055	T/C	0.045	2.311	0.365	2.3×10^{-10}	2.311	0.366	2.7×10^{-10}	SLX4; CREBBP
26	rs42083046	7,416,303	C/T	0.199	-1.268	0.195	8.6×10^{-11}	-1.268	0.196	1.0×10^{-10}	PRKG1
28	rs137496077	36,027,888	G/A	0.488	-0.917	0.162	1.4×10^{-8}	-0.917	0.162	1.5×10^{-8}	TSPAN14

¹Position = base pair positions in UMD3.1.²A1/A2 = effect allele/other allele.³Freq = frequency of A1 allele.⁴b = allele substitution effect; P = P-value from single-marker GWAS.⁵Subscript “j” indicates values from conditional and joint analysis.⁶Nearby gene = genes within 500 kb upstream and 500 kb downstream of the lead variants.

Table 6. Fertility index-associated GWAS loci in Nordic Red Dairy cattle

Chromosome	Top marker	Position ¹	A1/A2 ²	Freq ³	Single-marker analysis ⁴			Conditional analysis ⁵			Nearby gene ⁶
					b	SE	P	b _j	SE _j	P _j	
1	rs135868307	131,241,313	G/T	0.413	-1.198	0.202	2.9×10^{-9}	—	—	—	<i>FOXL2</i>
2	rs450253115	93,470,575	G/C	0.047	-2.559	0.450	1.3×10^{-8}	-2.787	0.453	7.6×10^{-10}	—
2	rs42490886	97,240,584	T/C	0.138	-1.498	0.279	8.1×10^{-8}	-1.646	0.281	4.7×10^{-9}	<i>PIKFYVE</i>
4	rs210258782	36,974,279	T/C	0.194	-1.618	0.238	1.0×10^{-11}	—	—	—	<i>SEMA3A</i> ; <i>SEMA3E</i>
6	rs110759619	39,633,323	G/A	0.481	1.373	0.189	3.3×10^{-13}	1.373	0.190	4.4×10^{-13}	—
6	rs379594438	95,384,366	G/A	0.055	2.381	0.421	1.6×10^{-8}	2.381	0.423	1.8×10^{-8}	<i>BMP2K</i>
8	rs470078356	87,251,892	T/C	0.013	-4.800	0.861	2.4×10^{-8}	—	—	—	<i>SPTLC1</i>
11	rs132823348	103,372,929	A/G	0.190	-1.343	0.242	2.7×10^{-8}	—	—	—	—
12	rs386105326	11,016,415	G/A	0.113	-2.336	0.308	3.2×10^{-14}	-2.336	0.310	4.6×10^{-14}	<i>SUGT1</i>
12	rs385475503/rs797826108	21,149,563	G/T	0.141	-4.087	0.290	4.9×10^{-45}	-11.123	0.445	8.0×10^{-138}	<i>WDFY2</i> ; <i>INTS6</i> ; <i>ATP7B</i> ; <i>ALG11</i>
12	rs384604017	29,738,986	T/C	0.285	-0.482	0.221	2.9×10^{-2}	9.110	0.464	7.0×10^{-86}	<i>B3GLCT</i> ; <i>RAPP2</i> ; <i>ALOX5AP</i>
12	rs432066150	29,790,646	C/T	0.274	0.734	0.224	1.0×10^{-3}	-5.561	0.359	3.6×10^{-54}	Same as rs384604017
12	rs377982349	30,453,718	G/A	0.291	-0.962	0.226	2.1×10^{-5}	3.602	0.316	4.7×10^{-30}	<i>HMGBI</i> ; <i>KATNAL1</i>
12	Chr12:31178612	31,178,612	C/T	0.128	-2.820	0.298	3.1×10^{-21}	-14.778	0.636	2.5×10^{-119}	<i>MTUS2</i> ; <i>SLC7A1</i>
12	Chr12:43320610	43,320,610	A/G	0.087	-2.424	0.338	7.1×10^{-13}	-2.424	0.340	9.4×10^{-13}	—
12	rs377982349	55,272,444	C/T	0.086	-1.944	0.348	2.3×10^{-8}	-1.944	0.349	2.6×10^{-8}	<i>SPRY2</i>
12	rs133220791	69,485,528	T/C	0.234	-1.374	0.230	2.2×10^{-9}	-1.374	0.231	2.5×10^{-9}	—
13	rs207566536	46,143,358	G/A	0.136	-1.794	0.277	1.0×10^{-10}	-1.794	0.279	1.2×10^{-10}	—
13	rs378749244	56,756,424	T/C	0.475	1.246	0.206	1.4×10^{-9}	1.246	0.207	1.6×10^{-9}	—
17	rs136268337	59,753,524	T/C	0.353	1.200	0.210	1.1×10^{-8}	—	—	—	<i>KSR2</i>
19	rs135743550	39,835,929	A/G	0.302	-1.275	0.216	3.5×10^{-9}	—	—	—	<i>SRCIN1</i>
25	rs382027875	13,426,672	T/C	0.055	-3.091	0.420	1.8×10^{-13}	—	—	—	<i>MRTFB</i>
26	rs481408336	35,317,193	A/G	0.061	2.147	0.363	3.3×10^{-9}	—	—	—	<i>ABLIM1</i>

¹Position = base pair positions in UMD3.1.²A1/A2 = effect allele/other allele.³Freq = frequency of A1 allele.⁴b = allele substitution effect; P = P-value from single-marker GWAS.⁵Subscript “j” indicates values from conditional and joint analysis.⁶Nearby genes = gene within 500 kb upstream and 500 kb downstream of the lead variants.

Table 7. Linkage disequilibrium (r) pattern among the top markers of chromosome 12 (Chr12) from Table 6

SNP	rs386105326	rs385475503	rs384604017	rs133494863	rs432066150	Chr12:31178612	Chr12:43320610	rs377982349	rs133220791
rs386105326	1								
rs385475503	0	1							
rs384604017	0	0.335	1						
rs133494863	0	-0.196	0.329	1					
rs432066150	0	0.358	0.393	-0.259	1				
Chr12:31178612	0	0	0.600	1	0.601	1			
Chr12:43320610	0	0	0.600	-0.236	0.601	0	1		
rs377982349	0	0	0	0	0	0	0	1	
rs133220791	0	0	0	0	0	0	0	0	1

to within-breed QTL discovery, which needs replication in independent cohorts of animals. Nevertheless, QTL identified in this study will be included in future releases of the custom SNP chip used for genomic evaluations; this will enable their validation in independent populations, and subsequent studies will elucidate the effect of these markers in prediction accuracy for female fertility in dairy cattle.

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ORCIDS

- Md Mesbah-Uddin <https://orcid.org/0000-0003-1846-0411>
 Bernt Guldbrandtsen <https://orcid.org/0000-0003-1764-135X>
 Aurélien Capitan <https://orcid.org/0000-0003-3185-0932>
 Didier Boichard <https://orcid.org/0000-0003-0361-2961>
 Goutam Sahana <https://orcid.org/0000-0001-7608-7577>